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Pretreatment levels of urinary deoxypyridinoline as a potential marker in patients with prostate cancer with or without bone metastasis

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Objective To assess the predictive role of the bone markers alkaline phosphatase (ALP) and urinary deoxypyridinoline (DPD), as indicators of bone turnover, at baseline in patients with prostate cancer.

Patients, subjects and methods Urinary DPD, serum ALP and prostate-specific antigen (PSA) were evaluated in 23 patients with benign prostatic hyperplasia (BPH), 115 with prostatic carcinoma, of whom 21 had bone metastasis, and in 16 age-matched control subjects.

Results Patients with newly diagnosed prostate cancer and bone metastasis had a higher urinary excretion of DPD, and a higher serum PSA and ALP than had patients with BPH and those with prostate cancer but no metastasis. Receiver operating curve analysis for

PSA, ALP and DPD showed a significant discriminating ability for positive and negative bone scans ($P=0.0684$). However, from logistic regression of the combinations, only serum ALP was a significant independent predictor of bone metastasis in patients with prostate cancer.

Conclusion Serum ALP or urinary DPD are the best predictors of bone metastasis in patients with prostate cancer; further studies with more patients are required.

Keywords prostate cancer, bone metastasis, serum prostate-specific antigen, alkaline phosphatase, urinary deoxypyridinoline

Introduction

In many Western countries prostate cancer is the second most common cause of cancer death in men, and the incidence is increasing [1,2]. At initial presentation 40% of patients with prostate cancer have bone metastases [3]; these are predominantly osteoblastic. Bone is a dynamic structure and constantly in a state of turnover, a metabolic process termed remodelling. This includes degradation (bone resorption) mediated by the action of osteoclasts, and building (bone formation), mediated by the action of osteoblasts. Remodelling is required for the maintenance and overall health of bone, and is tightly coupled. In normal bone there is a constant balance between bone formation and bone resorption. In abnormal situations of bone metabolism this process becomes uncoupled and, when resorption exceeds formation, results in a net loss of bone.

The introduction of new biochemical markers of bone metabolism has revealed various aspects of benign and malignant bone diseases. Of the organic matrix in bone,

≈ 90% contains type 1 collagen, a triple helical protein [4]. Type 1 collagen of bone is cross-linked by specific molecules that provide rigidity and strength. Crosslinks of mature type 1 collagen in bone are the pyridinium crosslinks, pyridinoline and deoxypyridinoline (DPD) [5]. DPD is formed by the enzymatic action of lysyl oxidase on the amino-acid lysine, is released into the circulation during the bone resorption process and represents, to a greater or lesser extent, the activity of metastases. DPD is excreted unmetabolized in urine; its uptake is unaffected by diet [6], allowing a suitable assessment of resorption.

Urinary DPD concentration has been used to estimate the degree of bone resorption. In the present study we investigated the utility of PSA, alkaline phosphatase (ALP) and urinary DPD as markers of bone metastasis in patients with prostate cancer.

Patients, subjects and methods

The study group comprised 138 men, referred to the urological outpatient clinic and who provided informed consent. Exclusion criteria were; bone, endocrine or chronic disorders, the use of medication known to

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influence bone metabolism (e.g. corticosteroids, GnRH analogues, anticonvulsants, thyroid medications or heparins). Patients in the study group had BPH or prostate cancer, the latter being clinically confined to the prostate (T1–2) or locally advanced (T3–4) or with bone metastases.

Patients with prostate cancer underwent bone scintigraphy to diagnose or exclude bone metastasis; 3–4 h after an intravenous injection with 700 MBq ^{99m}Tc -methylene diphosphonate, anterior and posterior whole-body images were obtained. Detailed images were acquired when considered necessary. According to the bone-scan results, the patients were divided into those with or with no bone metastases.

The control group of men, in which only DPD and creatinine were measured in the urine, comprised 16 presumably healthy young men aged 20–50 years. As these men were not part of a screening programme, no DRE or other laboratory results were known.

The level of serum PSA and ALP was determined in all patients. Blood samples were collected using a standard venipuncture technique and serum separated after centrifuging blood at 4°C and 1500 *g* for 10 min; samples were then stored at –20°C until analysis. Urine samples for the determination of DPD crosslinks were obtained from the first or second morning void, collected on the day of the bone scan. In the control group the urine samples were collected within 2 weeks of their outpatient department visit. The urine specimens were immediately frozen at –20°C. The concentration of DPD was expressed relative to the concentration of urinary creatinine ($\mu\text{mol}/\text{mol}$ creatinine) to control for differences in body mass and urine dilution.

Serum ALP was analysed according to the manufacturer's instructions on a Synchron LX 20 autoanalyser and reagents (Beckman Coulter Instruments Inc., Fullerton, CA, USA); the reference value for normal subjects is 40–130 U/L.

PSA in serum was determined using a chemiluminescent assay on an Immulite 2000 automatic immunoassay system, according to the manufacturer's instructions (DPC, Breda, The Netherlands); the threshold value used was 4 $\mu\text{g}/\text{L}$.

The urinary concentration of free deoxypyridinoline was determined using a chemiluminescent enzyme immunoassay according to the manufacturer's instruction (Immulite Pyrilinks-D, a solid-phase, chemiluminescent enzyme immunoassay for use with the Immulite I Automated Analyser, and designed for the quantitative measurement of DPD in urine; DPC). The normal reference range for urinary DPD (normalized to creatinine level) was 2.3–5.4 nmol/mmol creatinine. Urinary creatinine concentrations were determined using the Jaffe rate method (Synchron LX 20).

All analyte levels are presented as the median (range), with the significance of differences between the control and the different groups of patients assessed using the Mann–Whitney *U*-test, and $P < 0.05$ considered to indicate statistical significance. The relative effectiveness of the diagnostic tests was assessed by plotting the true-positive (sensitivity) vs false-positive (1-specificity) ratios on a ROC curve. This analysis is particularly useful for comparing two diagnostic tests when the threshold for a positive or negative test has not been clearly defined. Areas under the ROC curve were compared. To evaluate the independent contribution of PSA, ALP and DPD in predicting bone metastasis, correlation coefficients were calculated, and followed by logistic regression analysis. For this analysis all values were transformed onto a logarithmic scale and linear regression analysis used, with $P < 0.05$ considered significant.

Results

Twenty-three patients had clinically diagnosed BPH, later confirmed pathologically from TURP material; 115 patients had prostate cancer, histologically confirmed after biopsy and/or surgery (TURP, open prostatectomy or radical prostatectomy). The characteristics of the patients are shown in Table 1. The patients with BPH had normal values of PSA and, as expected, those with prostate cancer had an elevated PSA level. The median PSA level differed significantly between patients with and with no bone metastasis, although there was some overlap in the PSA values between the groups.

Table 1 also gives the values of ALP and DPD for the three patient groups and the controls. The urinary excretion of DPD relative to creatinine and serum ALP was significantly greater in the 21 patients with bone metastasis than in those with BPH or with no bone metastasis ($P < 0.001$). The urinary excretion of DPD was similar in the last two groups. Similarly patients with prostate cancer and bone metastasis had a significantly higher level of PSA ($P < 0.001$) and ALP ($P < 0.001$) than those with no metastasis (Table 1).

The relative effectiveness of PSA, ALP and DPD/creatinine in urine for discriminating between T3–4 prostate cancer with and without bone metastasis is shown in the ROC curves (Fig. 1). The sensitivity appeared to be comparable, at 0.643 for PSA, 0.792 for DPD/creatinine and 0.815 for ALP. The relationships among PSA, ALP and DPD in patients with prostate cancer are shown in Fig. 2. Although all correlation coefficients were significantly positive ($P < 0.005$), the correlation between PSA and ALP was low ($r = 0.268$, Fig. 2a), and between PSA and DPD only moderate ($r = 0.317$, Fig. 2b). The strongest relationship was between ALP and DPD ($r = 0.635$, Fig. 2c). Logistic

Table 1 Characteristics of the patient groups and the levels of the markers

Variable	BPH	Prostate cancer			Controls
		T1–2	T3–4	Bone metastasis	
No. of patients	23	46	48	21	16
Median (range)					
Age, years	68 (48–87)	73 (52–80)	69 (36–89)	74 (48–91)	–
PSA, µg/L	3.05 (0.2–11.5)*	17 (0.5–328)†	39.2 (0.1–1053)	81 (0.74–3063)	–
ALP, U/L	75 (43–185)	73 (34–313)	68 (44–204)	152 (29–1190)‡	–
DPD/creatinine, µmol/mmol	6.2 (3.94–13.3)	6.0 (1.9–300)	6.3 (4.8–50.0)	14.09 (4.6–300)‡	4.42 (2.96–6.58)

$P < 0.001$, *BPH vs prostate cancer with no metastasis; †prostate cancer with no metastasis vs prostate cancer with metastasis (Mann–Whitney U-test). ‡Control group vs BPH vs prostate cancer with no metastasis vs prostate cancer with metastasis (Kruskal–Wallis test).

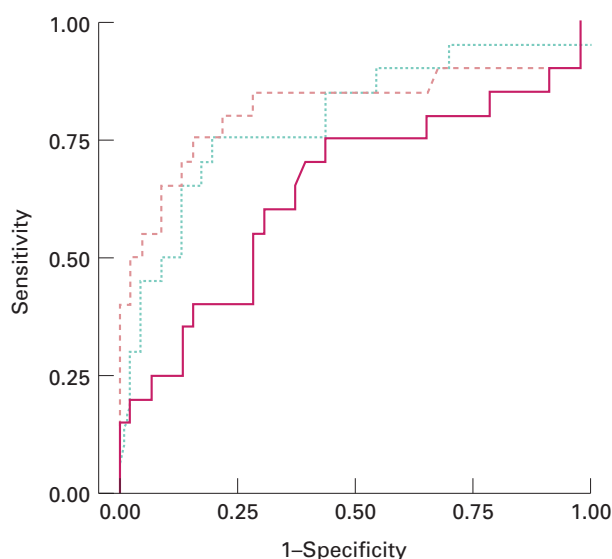


Fig. 1. ROC curves for PSA (red, solid), ALP (light red, dashed) and DPD (green, dotted) in patients with T3–4 prostatic carcinoma with and without bone metastasis. Areas under the ROC curves suggested that sensitivity for all markers was comparable and not significantly different from each other.

regression showed that in combination, only ALP had a significant independent contribution to predicting the presence of bone metastasis, the odds ratios (95% CI) being 1.04 (0.685–1.593) for PSA, 5.88 (1.156–29.873) for ALP and 1.92 (0.460–8.019) for DPD/creatinine. Table 2 gives the sensitivity, specificity, positive predictive value, false-positive and false-negative values for ALP, DPD and PSA at various thresholds for the individual patients.

Discussion

Bone metastases are a major cause of morbidity in patients with prostate cancer, causing subsequent significant clinical problems, including pain and pathological fractures. Therefore detecting bone metastasis is crucial for the staging, treatment and follow-up of prostate cancer. Bone scintigraphy, used in the initial staging and during the follow-up of prostate cancer, has been the most sensitive but not the most specific method to detect bone metastasis. 'Hot spots' on a bone scan are not always metastases, but can be associated with benign

Table 2 Sensitivity, specificity, positive predictive value (PPV), false-positives and false-negatives for ALP, DPD/creatinine and PSA at various thresholds

Marker/threshold	Sensitivity, %	Specificity, %	PPV, %	False-positive, n	False-negative, n
ALP (N=69)					
60	91	31	37	33	2
80	81	71	55	14	4
100	71	88	71	6	6
DPD/creatinine (N=66)					
6	90	37	38	29	2
7	85	57	46	20	3
10	65	83	62	7	8
PSA (N=69)					
10	86	13	30	42	3
20	81	21	31	38	4
40	76	50	40	24	5
60	62	69	46	15	8

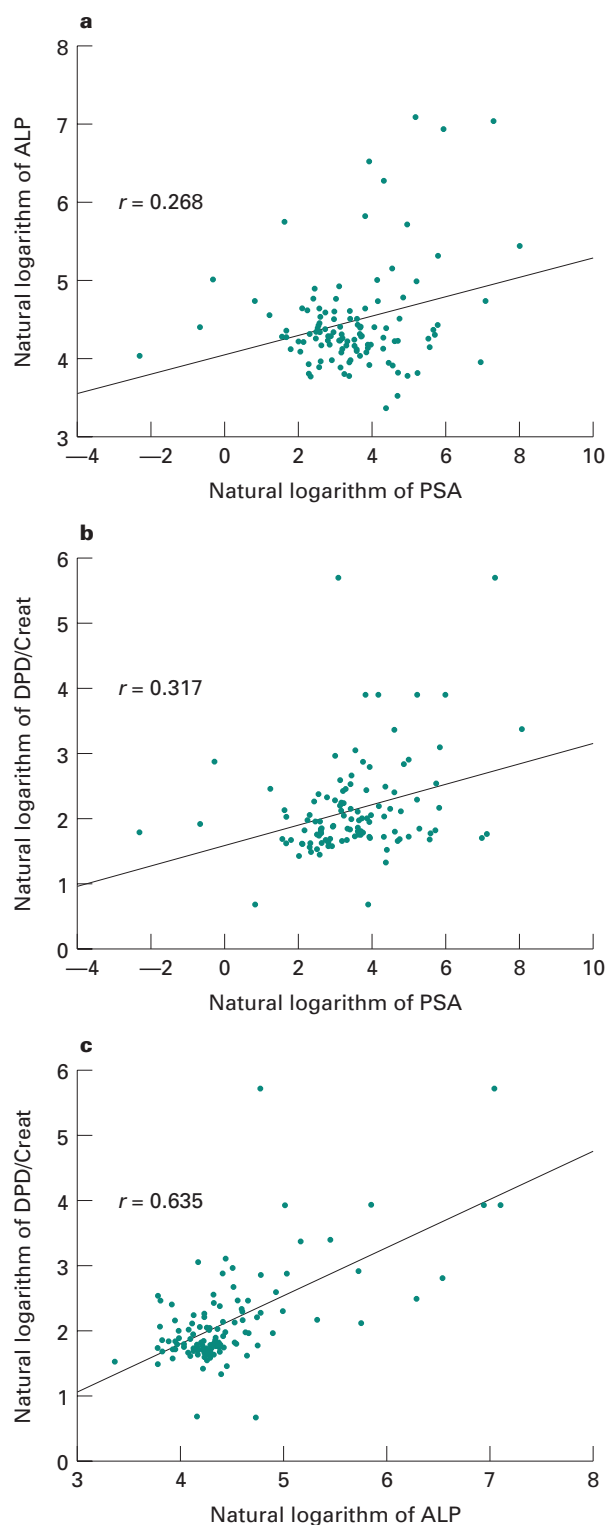


Fig. 2. Relationships between a, PSA and ALP ($r=0.268$), b, PSA and DPD/creatinine ($r=0.317$), and c, ALP and DPD/creatinine ($r=0.635$). All values are expressed on a logarithmic scale. All correlation coefficients are significantly different from 0 at $P<0.005$.

diseases, e.g. degenerative changes or trauma. Furthermore, bone scans are expensive and time-consuming. More sensitive and specific serum markers to discriminate between patients with and without bone metastasis from prostate cancer would be valuable for the clinician, particularly in the early diagnosis and additional treatment of advanced prostate cancer.

PSA is the most useful marker for determining the extent of prostate cancer; in patients with a PSA level of $\leq 10 \mu\text{g/L}$ an initial bone scan is, according to published studies, usually unnecessary [7,8]. PSA is not specific for bone metastases and is affected by other metastatic burdens. Moreover, in poorly differentiated carcinoma, there is not always a correlation between tumour volume and the serum PSA value [9]. PSA expression is regulated by androgen and after hormonal manipulation 34% of patients have a normal PSA, despite progression of bone metastasis [10].

In the present study, patients with bone metastasis had significantly greater levels of DPD in urine than had patients with no bone metastasis or with BPH ($P<0.001$). However, the combination of ALP and DPD appeared to be the most powerful predictor of bone metastasis (Table 2). This result refocuses attention on serum ALP as an important marker of metastatic disease. Urinary DPD may provide a useful marker to supplement ALP and PSA in evaluating bone scan results and the response to hormonal therapy [11,12].

In conclusion, the detection of possible bone metastasis is essential in the treatment of patients with prostate cancer. Serum ALP and PSA combined with the assay of urinary DPD may provide valuable additional indicators of metastases to the bone in untreated patients, and in monitoring the efficacy of therapy [11,12]. Additional studies with more patients and information during the follow-up are needed.

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Abbreviations: **DPD**, deoxypyridinoline; **ALP**, alkaline phosphatase.